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DEPARTMENT OF
ANIMAL SCIENCES

March 24, 1999

Dildree Ivery, Grant Specialist
Office of Naval Research
Resident Representative
Federal Building, Room 208
536 South Clark Street
Chicago, IL 60605-1588

Dear Dr. Ivery:

I have enclosed a final technical report for a NASA grant titled "Hypogravity's Effect on the Life Cycle of Japanese Quail" (NAG 2-1001).

Sincerely,

P. Y. Hester

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Professor of Animal Sciences

CC: 202A-3

CASI

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Calcium utilization by quail embryos during activities preceding space flight and during embryogenesis in microgravity aboard the orbital space station, MIR

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ABSTRACT A series of studies were conducted to determine the effect of activities preceding space-flight and during space-flight on quail embryonic development. While the overall development of the quail embryos was evaluated, the report presented herein, focused on calcium utilization or uptake from eggshells by developing embryos during incubation in space and on earth. In the pre-space trials, fertilized quail eggs were subjected to pre-flight dynamics including forces of centrifugation, vibration, or a combination of vibration and centrifugation prior to incubation for 6 or 16 days. In another trial, fertile quail eggs were tested for survivability in a refrigerator stowage kit for eggs (RSKE) which was subsequently used to transport the eggs to space. Eggs in the RSKE were subjected to shuttle launch dynamics including G force and random vibration profiles. In the space- flight trials, 48 fertile quail eggs were launched on space shuttle Flight STS-76 and were subsequently incubated in a Slovakian incubator onboard space station, MIR. Two sets of ground controls each with 48 fertile eggs with and without exposure to launch dynamics were initiated 5 days post-launch. There was a laboratory control (incubated in Lyon RX2 incubator at 37.5° C) and a synchronous control (incubated in Lyon RX2 incubator at 39-40° C), which simulated the temperature of the space-flight incubator. Following space-flight trials, post-flight trials were conducted where quail eggs were incubated in Lyon RX2 or Slovakian incubators under various temperatures with or without launch dynamics. Eggshells from all study trials were retrieved and analyzed for calcium content to determine if its utilization by developing quail embryos was affected by activities preceding space-flight or during incubation in space under microgravity.

Results from the pre-flight and post-flight showed that pre-flight activities and shuttle launch dynamics had no effect on calcium uptake from the eggshell by developing embryos. However, calcium uptake from the eggshell by developing embryos incubated in microgravity was impaired by 12.6% when compared to embryos incubated on earth under laboratory control environment. This impairment was unlikely due to factors other than microgravity. In general, calcium utilization by developing embryos increased with age of incubation with the most increase occurring at day 16 of incubation.

Key Words: calcium utilization . quail embryos . space . microgravity

EGG ROTATION DURING AVIAN EMBRYOGENESIS[1]

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Running Head: ROTATION OF AVIAN EGGS

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Hester, P. Y., J. I. Orban, V. Sabo, and K. Boda. Egg rotation during avian embryogenesis. *J Grav. Physiol.* 1997 (submitted) ___ Exposing early stage chicken embryos to the space environment caused death, while chicken embryos launched into microgravity in later stages developed normally. Quail embryos have successfully completed embryogenesis in orbit, although success rate has been low compared to earth-bound controls. Microgravity's role in the death of the embryos will not be known until a centrifuge is employed in orbit. Since avian eggs in microgravity were not turned, or were turned too infrequently or inadequately, the objectives of the current ground-based study were to determine the effects of frequency and orientation of turning on embryogenesis. Quail embryo viability was not affected by incubating eggs horizontally with daily rotation (4X) as compared to vertical orientation during incubation with hourly rotation. A decrease in frequency of egg rotation caused a concomitant linear decrease in hatchability for both quail and chicken eggs ($p<0.01$). The hatchabilty of fertile chicken eggs were more adversely affected by lack of egg rotation than were quail eggs ($p<0.05$). Gas exchange and nutrient distribution may occur more readily in unturned quail eggs as compared to chicken embryos because of the smaller quail egg size. The distance between the settling blastoderm relative to the shell surface is shorter for quail than for chicken embryos which may increase the probability of survival for quail as opposed to chicken embryos.

Key Words: Egg Turning, Axis of Rotation, Chicken, Quail, Embryo

360 day report
Hypogravity's Effect on the Life Cycle of Japanese Quail – Patricia Y. Hester, Ph.D.
Purdue University, West Lafayette, IN

IV. DISCUSSION

A. STATUS OF DATA ANALYSIS

Five additional ground control groups were dissected May 26 through June 5, 1997 at Ames Research Center. Eggshell mineral analysis of these additional ground controls are currently in progress.

B. FINAL RESEARCH FINDINGS

Slovakian, Russian, United States, and Japanese scientists have conducted experiments on the effects of the space environment on avian development. A common result of all of these flight experiments is that avian viability in space is lower than the ground based controls, and this is especially evident with chicken embryos during the early stages of embryogenesis. Future experiments in which avian eggs are centrifuged in space will determine if microgravity was contributing to the premature death of some of the avian embryos.

Other factors of the space environment besides microgravity should also be considered as possible causes of the premature death of some of the avian embryos. For example, avian embryos studied thus far in microgravity were either not turned or may have been improperly rotated. Based on studies conducted on earth, avian eggs need to be turned a minimum of three times per day and is critical during the first seven days of incubation (4, 16). The United States experiment (STS-29) with chicken embryos in microgravity did not employ turning; all of the younger chicken embryos (2 to 7 days) died while the older embryos (9 to 14 days) developed normally (7, 10). Although Japanese scientists had astronauts manually shake or tilt the chick egg containers with the air cell in the upward position three times "to the left and to the right from the 9 to 3 o'clock direction" twice daily with a return to their original orientation, younger chicken embryos also faced premature death in microgravity (15). Japanese quail have experienced more success with the earlier stages of embryogenesis than chickens though rates of development and hatch have been lower compared to synchronous and laboratory controls (8, Table 1). Embryo viability of the ground base control groups of the current study (NASA 2 / MIR 21) showed that lack of turning adversely affected embryo viability (Synch 2, Table 1).

Table 1. Viability of flight (NASA 2 / MIR 21) and ground based control avian embryos

Treatment	Number of times eggs were turned	Orientation of eggs	Mean embryo viability (%)
Flight	Free floating	Random	59
Lab 1	1X/hr	Horizontal	91
SYNCH 1	1X/hr	Horizontal	74
SYNCH 2	**None**	**Random**	**55**
SYNCH 3	3x/day*	Random	85
LAB 2	1x/hr	Horizontal	75
LAB 3	3x/day	Vertical	88
LAB 3 - HI	3x/day	Vertical	81

* Turning by tray rotation. Eggs moved at random as a result of tray rotation (Steve Piert, personal communication).

Although quail eggs on MIR were not turned, they were free floating during incubation; therefore, it is possible that vibrations from the space station could have allowed for some movement of the eggs. Randles and Romanoff (13) have reported that the shaking of eggs improves hatchability as compared to static eggs, but is no substitute for turning.

On earth, failure to turn the eggs in the early stages of embryogenesis retards embryonic growth and decreases hatchability (4). Rotation of eggs is needed to facilitate respiratory exchange between the embryos and the pores of the shell and to promote nutrient distribution. In chicken eggs, lack of turning reduces the expansion of the area vasculosa which would interfere with nutrient distribution (5). Earth-based studies have shown that turning produces changes in the density of the albumen, yolk, and subembryonic fluid that promotes buoyancy of the yolk sac (2, 6). Rotation of eggs ensures an early transfer of water from albumen to yolk, thus creating by mid-incubation, an albumen residue that is high in density and low in volume. The yolk density of rotated eggs decreases, thus increasing the buoyancy of the yolk sac which facilitates its rise towards the inner shell membrane. The movement of the yolk closer to the shell membrane facilitates respiratory exchange. Static eggs would not allow embryos to come in close proximity to the pores of the shell resulting in inadequate oxygen and carbon dioxide exchange. Turning also promotes the transfer of yolk nutrients such as protein and lipids to the subembryonic membrane. Whether or not microgravity influences any of these changes due to egg rotation is unknown. Since there is no weight in microgravity, turning eggs in the space environment may have little effect on the relative density changes of the yolk and albumen that normally occurs with avian embryos developed on earth. Without density changes in the albumen and yolk, perhaps developing embryos in microgravity are deprived of oxygen because of its distance from the pores of the shell or due to the inadequate development of the chorioallantoic membrane. On the other hand, rotation of eggs in microgravity should not be detrimental to the embryos and may actually increase the number of viable embryos by enhancing nutrient distribution from the yolk to the developing embryo by promoting the development of the chorioallantoic membrane.

The objectives of the current study were two-fold. First, we conducted a comparative study on the effects of turning frequency on the viability of quail and chicken embryos. The possibility exists that chicken eggs are more susceptible to premature death due to lack of egg rotation than quail eggs, which could offer an explanation of quail's increased survivability in the space environment. Secondly, new hardware for avian development in microgravity is currently being developed with a centrifuge and turning device in which eggs are to be rotated around their longitudinal axis. Such an orientation allows the force of gravity created by the centrifuge to act perpendicular to the longitudinal axis of the egg similar to natural incubation. The advantage in orienting the egg so that the gravity vector is perpendicular to the longitudinal axis during centrifugation is to minimize the *g* force gradient across the axis of the egg. Therefore, the second objective was to determine the effect of axis of rotation and frequency of turning on the viability of quail embryos.

Methods of Experiment 1. Eighteen days prior to incubation, eggs from a 26-week-old hypodynamic strain of Japanese quail (Laying Line 01, Ivanka pri Dunaji), and eggs from 51-week-old Bovan White Leghorn hens were collected daily and stored in a cooler at 13° C. Eggs were incubated in a Jamesway 252 incubator (James Manufacturing, Co., Fort Atkinson, WI 53538) where temperature and relative humidity were maintained at 37.5°C and 61%, respectively. Immediately prior to initiation of incubation, eggs were examined for cracks in the shell via candling. Eggs with intact shells were set in the incubator tray in a vertical position with the large end up. Five turning treatments were employed in which eggs were rotated 0, 1, 2, 3,

and 4 times daily at equally spaced intervals for the first seven days of incubation. Eggs subjected to no turning remained in a vertical position, while eggs that were rotated over a 90° arc were alternated 45° from vertical to reverse 45°. On day 8 of incubation, all trays were disengaged so as to terminate egg rotation. Eggs were positioned in the vertical position with the large end of the egg up from day 8 of incubation until transfer to hatching baskets. For hatching, quail and chicken eggs were placed horizontally in pedigree baskets and transferred to another Jamesway 252 incubator on day 15 and 19 of incubation, respectively. On days 18 and 21 of incubation for quail and chicken eggs, respectively, the number of chicks which hatched was assessed. Unhatched eggs were opened to determine fertility, and % early, middle, and late deads (11).

Data were subjected to an analysis of variance with two split plots, the species of bird and the age of the eggs. Trays within turning treatments served as the error term for the main effect of turning treatment. Turning treatment, species of bird, and age of the eggs were considered fixed effects, whereas trays within turning treatments were considered random. Orthogonal polynomial comparisons were used to examine turning treatment effects. Student Newman-Keuls' multiple range test was used to partition differences among means for significant turning treatment by species interactions. Percentage data were transformed to arc sine. Statistical patterns were the same for both transformed and untransformed data; therefore, for ease of interpretation, only untransformed results will be presented (1, 14).

Methods of Experiment 2. Seven days prior to incubation, eggs of the hypodynamic strain of Japanese quail (Laying Line 01, Ivanka pri Dunaji), 9 weeks of age, were collected daily, marked with the date collected, and stored in a cooler at 13°C. On the day the eggs were set, each egg was candled to exclude any eggs with cracked shells. Using a water resistant marker, a single dot was placed on the equator of each selected egg for the purpose of orientation during turning. Eggs were set horizontally in hatching baskets and manually turned 180° around the long axis in opposite direction at equally specified intervals of 0, 1, 2, 3, and 4 times daily. To prevent eggs from rolling while removing and returning eggs to the Jamesway 252 incubator, the bottom of the baskets were lined with plastic hardware cloth with mesh size of 2.5 cm^2. A control turning treatment included eggs set vertically with the large end up, and rotated automatically each hour over a 90° arc as described in Experiment 1. Another control, referred to as tray relocation, was included as an additional treatment to account for movement and vibrations that were incurred during tray removal and return to the incubator. Specifically, tray relocation involved the repositioning to a new tray level twice daily at 12 hour intervals without any rotation of the eggs. It differed from the 0 turning treatment in that 0 turning treatment eggs were left untouched and were not removed from their tray position in the incubator until time of transfer to the hatcher. Temperature and humidity were recorded twice daily before 0800 hour and before 1600 hour. Temperature was maintained at 37.5°C and relative humidity at 61%. On Day 8 of incubation, eggs were no longer turned. On day 15 of incubation, control eggs incubated in a vertical position were transferred to hatching baskets; and lids were placed on all hatching baskets. On day 18 of incubation, the number of chicks which hatched was assessed along with fertility and embryonic mortality pattern as described in Experiment 1. Data were analyzed as a one-way analysis of variance. Percentage data were transformed to arc sine, but only untransformed data will be presented for reasons already described in Experiment 1. Student Newman-Keuls' sequential range test was used to partition differences among means (14).

Results of Experiment 1. A decrease in frequency of egg rotation caused a concomitant linear decrease in hatchability for both quail and chicken eggs as indicated in Table 2 for hatch of set and hatch of fertile ($p<0.01$).

Table 2. The effect of egg rotation on hatchability of quail and chicken eggs (Experiment 1)

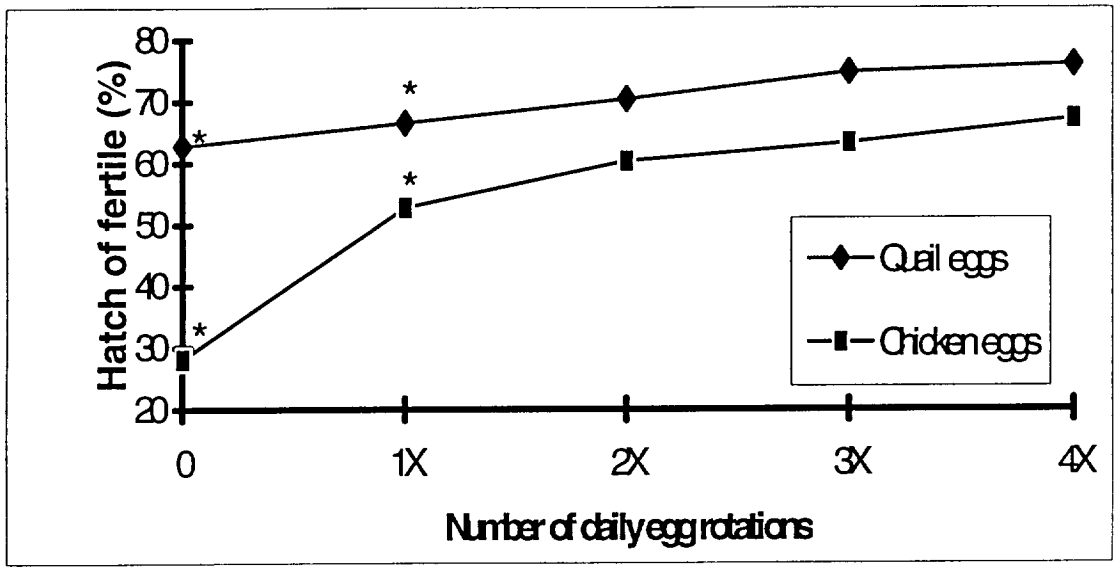
	Hatch of set		Hatch of fertile		Infertile	
Turning Treat.[1]	Quail eggs	Chicken eggs	Quail eggs	Chicken eggs	Quail eggs	Chicken eggs
			%			
0	63	26	63*	28*	0	4
1X	66	49	67*	53*	1	7
2X	69	56	70	60	1	8
3X	75	58	75	63	0	7
4X	76	64	76	67	0	4
SEM	4		3		2	

[1] Eggs were incubated vertically. For turning treatments of 1X, 2X, 3X, and 4X, eggs were rotated over a 90° arc at intervals of 1, 2, 3, or 4 times per 24 hr period for the first 7 days of incubation. Each turning treatment was replicated twice for a total of 72 eggs per treatment per species.

* Significant turning treatment by species of egg interaction ($p<0.05$).

Fertile chicken eggs were more adversely affected by lack of egg rotation than quail eggs as indicated by the significant turning treatment by species of egg interaction ($p<0.05$, Fig. 1). While chicken eggs had lower rates of hatch than quail eggs ($p<0.001$) for all turning treatments, no egg rotation at all or turning eggs only once per day caused an even more severe reduction in hatchability (Fig. 1).

Figure 1. The effect of number of daily egg rotations on hatchability (hatch of fertile eggs set) of quail and chicken eggs. Eggs were turned during the first seven days of incubation and rotated over a 90° arc (Experiment 1). The interaction of turning treatment by species of bird was significant at a $p<0.05$. Asterisks indicate significant differences between species with 0 and 1X daily egg rotations. The SEM was 3.3.



The percentage of mid ($p<0.02$) and late deads ($p<0.06$) showed a significant linear response to egg rotation (Table 3). Less frequent turning caused an increase in mid and late deads for both quail and chicken eggs. Static chicken eggs which were never turned experienced a 21 % incidence of early deads as compared to a 7 % incidence in the unturned quail eggs (turning treatment by species interaction, $p<0.08$, Table 3).

Table 3. The effect of egg rotation on embryonic mortality of quail and chicken eggs (Experiment 1)

	Early dead		Mid dead		Late dead	
Turning treat.[1]	Quail eggs	Chicken eggs	Quail eggs	Chicken eggs	Quail eggs	Chicken eggs
	%					
0	7	21	6	7	25	42
1X	10	4	0	4	23	35
2X	8	3	2	4	20	29
3X	9	10	3	0	14	25
4X	3	7	0	0	21	25
SEM	3		3		4	

[1] Eggs were incubated vertically. For turning treatments of 1X, 2X, 3X, and 4X, eggs were rotated over a 90° arc at intervals of 1, 2, 3, or 4 times per 24 hr period for the first 7 days of incubation. Each turning treatment was replicated twice for a total of 72 eggs per treatment per species.

Results of Experiment 2. Hatchability of unturned horizontally incubated quail eggs was significantly reduced when compared to eggs incubated horizontally and rotated four times daily (hatch of set $p<0.02$ and hatch of fertile, $p<0.03$, Table 4). Turning horizontally incubated eggs 180° around the long axis at more frequent intervals resulted in a concomitant increase in hatchability. Horizontal orientation of eggs as compared to vertical orientation of eggs rotated hourly had no effect on hatchability (Table 4). Eggs subjected to tray relocation, which repositioned the baskets to a new tray level twice daily without egg rotation or movement, had hatch rates similar to eggs which were never turned (0 turning treatment, Table 4). The embryonic mortality pattern showed that as eggs were turned less frequently, the incidence of late deads increased ($p<0.04$, Table 4). Turning treatment significantly affected the incidence of early deads ($p<0.05$, Table 4), but due to an unusually high incidence of early deads among eggs incubated vertically and rotated hourly (19%, 24X), no consistent pattern was evident. The percentage of mid deads and infertiles were not affected by turning treatment.

Table 4. The effect of turning and axis of rotation on hatchability and embryonic mortality pattern of quail eggs (Experiment 2)

Turning Treatment[1]	Hatch of set	Hatch of fertile	Infertile	Early dead	Mid dead	Late dead
			%			
Tray relocation	48[b]	52[b]	8	13[ab]	0	35[ab]
0	50[b]	54[b]	7	3[b]	5	39[a]
1X	53[b]	59[ab]	10	9[ab]	3	30[ab]
2X	52[b]	60[ab]	13	8[ab]	8	23[ab]
3X	62[ab]	68[ab]	8	9[ab]	1	22[ab]
4X	76[a]	79[a]	4	4[b]	0	17[ab]
24X[2]	67[ab]	71[ab]	6	19[a]	0	10[b]
SEM	4	4	3	3	3	5

[1] Eggs for treatments on tray relocation, 0, 1X, 2X, 3X, and 4X were incubated horizontally. For treatments of 1X, 2X, 3X, and 4X, eggs were turned 180° around the long axis in opposite direction at specified intervals for the first seven days of incubation. Each turning treatment was replicated twice for a total of 84 eggs per treatment.

[2] Eggs were incubated vertically and rotated over a 90° arc every hour for the first seven days of incubation.

[a,b] Values within a column with no common superscript differ significantly ($p<0.05$).

C. CONCLUSIONS

Eggs in commercial incubators are set with the small end down. They are turned 45° from vertical where they remain at that position for an hour or longer before rotation is again automatically activated to rotate eggs to the opposite position, i.e., reverse 45° from vertical (12). Eggs incubated under commercial conditions do not need to be turned beyond the first week of incubation, but because different age eggs are intermingled within the incubator, they are all turned together (11). The brooding hen, however, incubates her eggs in a nest with the eggs oriented in a horizontal position, though the curvature of the nest and the shape of the egg allows the large end to be slightly higher than the small end of the egg. She frequently turns her eggs

using her body as she settles on the nest as well as her beak to move the eggs under her (12). The results of the current study suggest that quail can successfully hatch from eggs incubated artificially in a horizontal position if turned 180° around the long axis in opposite directions at least 4 times within a 24 h period. Turning eggs only one or two times during a 24 hour period, either in a vertical or horizontal position, will cause a decrease in hatchability. Hatch rates of quail eggs as a result of tray relocation provided similar results as no turning at all (Table 4). Similarly, tilting eggs and returning them to their original position as described by Suda *et al.* (15) in their incubation of chicken eggs in microgravity may be inappropriate turning. Buhr (3) studied the effect of tilting on hatchability instead of turning chicken eggs during incubation. Buhr used both the vertical and horizontal positioning. Eggs were tilted on a 45° arc, or horizontally 180°, and then they were returned to their original setting orientation. Buhr concluded that tilting instead of turning depressed hatchability regardless of setting orientation. Depressions were cumulative and additive with significant depression for all eggs tilted during the first week of incubation. There were diminished effects during the second and third weeks of incubation.

Chicken eggs were more adversely affected by lack of turning than quail eggs (Fig. 1). Since quail eggs are about one-third the size of chicken eggs, the quail blastoderm, which normally floats to the top surface of the yolk during early embryogenesis, is not as far from the surface of the shell as are chicken blastoderms. Rotation of eggs is needed to facilitate respiratory exchange between the embryos and the pores of the shell (6). Static eggs will not allow embryos to come in close proximity to the pores of the shell resulting in inadequate oxygen and carbon dioxide exchange. The shorter distance between a settling unturned blastoderm and shell surface of quail may make it less susceptible to embryonic death. Turning is also needed to promote nutrient distribution from the yolk and albumen to the developing embryo (6). Turning promotes the transfer of yolk nutrients to the subembryonic membranes. Static incubation deprives the embryo of adequate nutrient supply leading to starvation. In chicken eggs, but not quail eggs, lack of turning reduces the expansion of the area vasculosa which interferes with nutrient distribution (5). Again, because of the smaller size of quail eggs as compared to chicken eggs, nutrients may not have to travel as far to the growing embryo, thus explaining the less serious effect of lack of turning on hatchability of quail eggs.

Not all embryos of unturned chicken eggs die. In the present study, a hatch rate of 28.1% was observed for unturned chicken eggs (Table 2, hatch of fertile), results similar to that provided by North and Bell (11). Suda *et al.* (15) reported that 1 out of the 8 (12.5%) fertile chicken embryos exposed to the space environment during the first 7 days of embryogenesis survived landing and developed to the 16th day of incubation. The United States research team sent 16 younger chicken embryos into space and none survived landing (7). Perhaps a larger sample size than 24 could increase the probability of successfully completing chicken development in microgravity. However, as discussed in detail by Hullinger (10), other factors of the space environment, such as microgravity, radiation, etc., could have contributed to the premature death of the younger chicken embryos. Until incubation hardware with a turning device is flown in space, the role that egg rotation plays in the premature death of avian embryos exposed to microgravity will remain speculative. Likewise, gravity's role in early embryogenesis can only be validated by introducing artificial gravity though centrifugation in orbit.

REFERENCES

1. **Anderson, V. L. and R. A. McLean.** Design of Experiments. A Realistic Approach. Marcel and Dekker, Inc., New York, NY, 1974.

2. **Babiker, E. M. and G. K. Baggott**. Effect of turning upon the sub-embryonic fluid and albumen of the egg of the Japanese quail. *British Poult. Sci.* 33:973-991, 1992.

3. **Buhr, R. J.** Effect on hatchability of tilting instead of turning chicken eggs during incubation. *Poultry Sci.* 68: (Suppl.1):20 (Abstract), 1989.

4. **Deeming, D. C.** Characteristics of unturned eggs: Critical period, retarded embryonic growth and poor albumen utilisation. *British Poult. Sci.* 30:239-249, 1989.

5. **Deeming, D. C.** Failure to turn eggs during incubation: Development of the area vasculosa and embryonic growth. *J. Morphol.* 201:179-186, 1989.

6. **Deeming, D.C., K. Rowlett and K. Simkiss.** Physical influences on embryo development. *J. Exp. Zool. Suppl.* 1:341-345, 1987.

7. **Fermin, C. D., D. Martin, T. Jones, J. Vellinger, M. Deuser, P. Hester and R. Hullinger**. Microgravity in the STS-29 space shuttle Discovery affected the vestibular system of chick embryos. *Histol. Histopathol.* 11:407-426, 1996.

8. **Guryeva, T., O. A. Dadasheva, Y. Y. Shepelev, K. Boda and V. Sabo**. The quail embryonic development under the conditions of weightlessness. *Acta Vet. Brno,* Suppl. 6, 62:S25-S30, 1993.

9. **Hester, P. Y., M. E. McGinnis, J. C. Vellinger, M. S. Deuser and C. D. Fermin.** Avian embryogenesis in microgravity aboard shuttle STS-29; effect on shell mineral content and post-hatch performance. *Acta Vet. Brno,* Suppl. 6,62: S43-S47, 1993.

10. **Hullinger, R. L.** Embryogenesis aboard shuttle STS-29. *Acta Vet. Brno,* Suppl. 6,62: S17-S23, 1993.

11. **North, M. O. and D. D. Bell.** Chapter 8, Factors affecting hatchability. In: Commercial Chicken Production Manual, 4th ed. Van Nostrand Reinhold, New York, NY, 1990, pp. 103-134.

12. **Parkhurst, C. R. and G. J. Mountney.** Chapter 5, Incubation and hatchery management. In: Poultry Meat and Egg Production, Van Nostrand Reinhold, New York, NY, 1987, pp. 65-84.

13. **Randles, C. A. and A. L. Romanoff**. A preliminary study on the hatchability of chicken eggs subjected to shaking agitation. *Poult. Sci.* 33:374-377, 1954.

14. **Steel, R. G. D. and J. H. Torrie**. Principles and Procedures of Statistics. A Biometrical Approach. 2nd ed. McGraw-Hill Book Co., Inc., New York, NY. 1980.

15. **Suda, T., E. Abe, T. Shinki, T. Katagiri, A. Yamaguchi, S. Yokose, S. Yoshiki, H. Horikawa, G. Cohen, S. Yasugi and M. Naito.** The role of gravity in chick embryogenesis. *FEBS Letters*, 340:34-38, 1994.

16. **Wilson, H.R.** Chapter 9. Physiological requirements of the developing embryo: temperature and turning. In: Avian Incubation: Poultry Science Symposium No. 22, S. G. Tullet (ed.), Butterworth-Heinemann, Ltd. Surrey, United Kingdom, 1991, pp. 145-156.

D. INVESTIGATION RESULTS

Quail embryo viability was not affected by incubating eggs horizontally with daily rotation (4X) as compared to vertical incubation with daily rotation (24X). A decrease in frequency of egg rotation caused a concomitant linear decrease in hatchability for both quail and chicken eggs ($p<0.01$). Fertile chicken eggs were more adversely affected by lack of egg rotation than quail eggs ($p<0.05$). Gas exchange and nutrient distribution may be facilitated in unturned quail eggs as compared to chicken embryos because of its smaller egg size. The shorter distance between a settling unturned quail blastoderm relative to the shell surface may increase the probability of survival for quail as opposed to chicken embryos.

E. INVESIGATION APPLICATIONS

These data are supportive of the need to turn avian eggs on earth to ensure maximum viability of embryos.

V. BIBLIOGRAPHY

Hester, P. Y. and K. Boda, 1997. Egg rotation during avian embryogenesis. Am. Soc. Gravitat. Space Biol.(abstract, accepted).

Quail Eggshell Mineral Analysis
MIR 21 - NASA 2
180 Day Report

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OBJECTIVE:

This study was designed to determine the effect of space flight on quail embryo's utilization of mineral from the shell. Comparisons were made with laboratory controls (LAB -1) and synchronous controls (SYNCH -1).

METHODS:

Laboratory controls were incubated in a Lyon RX2 incubator at 37.5° C with egg rotation occurring hourly. Synchronous controls were also incubated in a Lyon RX2 incubator with hourly rotation, but temperature was maintained at 39° to 40° C to simulate the temperature of the Slovakian incubator during space flight. Neither LAB-1 or SYNCH-1 were subjected to acoustics, vibrations or *g* load. Embryos were placed in 4% paraformaldehyde fixative at 3, 7, 10, 14, and 16 days of incubation.

Quail eggshells obtained from this study were cleaned of egg contents and then rinsed with deionized water. The eggshells were air-dried at room temperature and stored in ziplock bags at room temperature. Following oven drying and ashing of the eggshells, the samples were analyzed for mineral (calcium, phosphorus and magnesium) contents in three sections, large end (A), middle portion (B) and small end (C). Each section was weighed in crucibles and dried at 100° C overnight. The samples were then weighed and ashed at 750° C overnight (8-12 hours). Ashed samples were weighed and digested with concentrated hydrochloric acid (HCl). One mL of purified water was added to each sample followed by 1 mL of concentrated HCl. The samples were allowed 5 to 10 minutes to completely dissolve. The dissolved samples were transferred to 15 mL tubes and diluted with purified water to a total volume of 10 mL. Two dilutions of samples were made for mineral analysis.

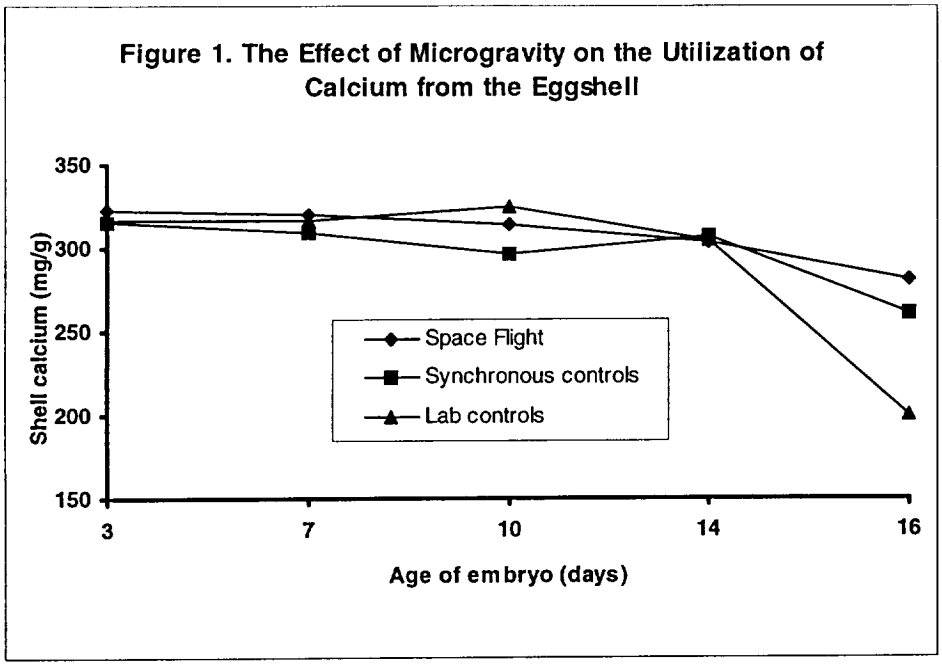
Calcium and phosphorus analyses were done using a 1:20 (vol./vol.) dilution, that is, 1 mL of the original dissolved sample from 10 mL volume was further diluted with purified water to a total volume of 10 mL. Magnesium was analyzed using the 1:10 dilution of the original sample. All samples were analyzed using the Inductively Coupled Plasma Atomic Emission Spectrometer (ICPAES) which has the capacity to analyze for multiple minerals at one time.

Analysis of variance was conducted on the data where treatment (flight *vs* controls), eggshell section (A, B, and C), and age of incubation were all considered fixed effects. Newman-Keuls multiple range test was used to partition differences among means.

RESULTS:

	Shell calcium (mg/g)	Shell magnesium (mg/g)
Treatment		
LAB 1	285[c]	4.0[a]
SYNCH 1	297[b]	3.8[b]
SPACE FLIGHT	314[a]	4.0[a]
Embryo Age		
Day 3	318[a]	4.1[a]
Day 7	315[a]	4.0[a]
Day 10	312[a]	4.1[a]
Day 14	306[a]	3.7[b]
Day 16	231[b]	3.8[b]
Eggshell section		
A	293	3.9[b]
B	291	3.8[b]
C	310	4.1[a]
Standard deviation	31	.4

Shell calcium was significantly affected by space flight treatment ($P < 0.02$) and the age of the embryo ($P < 0.0001$). Laboratory controls used more calcium from the shell than did the space flight and synchronous control embryos. Flight embryos used significantly less calcium from the shell when compared to both synchronous and laboratory controls. The longer embryos were allowed to incubate, the more calcium they utilized from the shell with an increase in shell calcium utilization occurring on the 16th day of incubation. The significant interaction between space flight treatment and age of the embryo ($P < 0.0001$) is shown in Figure 1. The differences among treatments became most apparent on the 16th day of incubation in which laboratory control embryos used more calcium from the shell than did the space flight and synchronous control groups. Shell calcium did not differ statistically between space flight and synchronous control embryos incubated to day 16. Shell calcium did not differ among eggshell sections.



Shell magnesium was significantly affected by treatment ($P < 0.0002$) and the age of the embryo ($P < 0.003$). Synchronous controls used more magnesium from the shell than did the space flight and laboratory control embryos. Embryos used more magnesium from the shell on day 14 and 16 of incubation than earlier ages of embryogenesis. The interaction between space flight treatment and age of the embryo was non-significant for shell magnesium. More shell magnesium was used from the middle (B) and large end (A) of the egg than the small end (C) of the egg.

Shell phosphorous concentrations were ten times higher than normal because of uptake of phosphorous from the fixative which contained a phosphate buffer. Results from the GAVEET study where fixation was not employed showed shell phosphorus levels at approximately 3 mg/g. Phosphorus levels of shells from the current study (MIR 21) were averaging around 30 mg/g; therefore, phosphorus analysis will be not be done on any future shells that have been in fixative. Shell calcium and magnesium concentrations did not appear to be affected by fixative.

DISCUSSION AND CONCLUSIONS:

Space flight conditions interfered with the 16 day-old quail embryos uptake of calcium from the shell. However, since synchronous control embryos at day 16 had shell calcium levels that did not differ statistically from space flight, the effect may have been due to the high incubator temperature (39° to 40° C) experienced during space flight as opposed to microgravity.

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EGG ROTATION DURING AVIAN EMBRYOGENESIS. Patricia Y. Hester[1] and K. Boda[2].[1] Dept. of Animal Sciences, Purdue University, West Lafayette, IN and [2]Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences, Ivanka pri Dunaji, Slovakia.

Exposing chicken embryos to the space environment caused death during earlier stages of embryogenesis, while older chicken embryos developed normally. Though success rate has been low compared to earth-bound controls, quail embryos have successfully completed embryogenesis in orbit. Microgravity's role in the death of the embryos will not be known until a centrifuge is employed in orbit. Since avian eggs in microgravity have not been turned, or turned too infrequently or inappropriately, the objectives of the current ground-based study were to determine the effects of frequency and orientation of turning on embryogenesis. Quail embryo viability was not affected by incubating eggs horizontally with daily rotation (4X) as compared to controls. A decrease in frequency of egg rotation caused a concomitant linear decrease in hatchability for both quail and chicken eggs ($p<0.01$). Fertile chicken eggs were more adversely affected by lack of egg rotation than quail eggs ($p<0.05$). Gas exchange and nutrient distribution may be facilitated in unturned quail eggs as compared to chicken embryos because of its smaller egg size. The shorter distance between a settling unturned quail blastoderm relative to the shell surface may increase the probability of survival for quail as opposed to chicken embryos.

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